*Original Article*

# Clinical and pathological findings in experimental brucellosis in pregnant rats

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**Abstract**
Background: To investigate the clinical findings and pathological changes in female Sprague-Dawley rats (SD) after experimental infection at late stage of pregnancy with *Brucella abortus* biotype 1 Korean isolate.
Methodology: Twenty-five rats were used and the rats were classified into two groups, an infected group (n=15) and a control group (n=10). At 18 days of gestation 500 microliters containing $1.0\times10^9$ colony forming unit (CFU) suspension of *B. abortus* biotype 1 Korean isolate in physiological saline solution was injected subcutaneously to each of 15 rats, and ten rats were injected with only 500 microliters of physiological saline. The SD rats were examined clinically and the spleen, lymph nodes, uterus and placenta were examined grossly and microscopically. Additionally these organs as well as the blood were cultured bacteriologically.
Results: There were no stillbirths, abortions or premature births in any of the SD rats. The gross signs of all the SD rats of the infected group included splenomegaly, metritis, enlargement of lymph nodes and placentitis. Moreover, *B. abortus* biotype 1 were detected in the organs of infected SD rats as well as in the blood. The microscopic signs of the SD rats of the infected group included infiltrations of macrophages, giant cells and engorged macrophages scattered in necrotic debris in the lymph nodes. In the spleen, there was diffuse congestion of the red pulp, diffuse infiltration of macrophages with increased giant cell numbers and prominent germinal centres. In the uterus, there was moderate, diffuse, but multifocally prominent accumulation of lymphocytes and macrophages in the superficial lamina. In the placenta, there were areas of necrosis in the periplacentomal chorionic epithelium.
Conclusions: It is concluded that the Korean pathogenic isolate *B. abortus* biotype 1 does not induce abortion in SD rats.

**Key Words**: *B. abortus* biotype 1, clinical findings and pathological changes, Sprague- Dawley rats, South Korea.

*J Infect Developing Countries* 2008; 2(3):226-229.

Received 31 December 2007 - Accepted 03 April 2008



## Introduction

Brucellosis, a zoonotic disease caused by members of the genus *Brucella*, affects both humans and animals such as cattle, sheep, goats, swine, and dogs [1-2]. Wildlife, including the rat, are also susceptible and may play a role in transmission to domestic animals and humans [2-3]. *Brucella abortus* has been isolated from rats and the rat infections occur in areas with large numbers of infected cattle, which suggests that the cattle are an important source of infection for rats [2-4].

Brucellosis is a major disease with a predilection to infect placenta and foetal membrane. The most common clinical features of brucellosis in animals are placentitis and abortion [5]. Guinea pigs and mice have been the principal animals used in laboratory experiments for brucellosis.

Pathogenic *B. abortus* biotype 1 has been isolated from cattle in different provinces of South Korea following extensive studies [6-8]. The purpose of the present study was to investigate the clinical findings and pathological changes in female Sprague-Dawley rats after experimental infection at late stage of pregnancy with *B. abortus* biotype 1.

## Materials & Methods

*Culture of* B. abortus

We used Korean isolate of *B. abortus* biotype 1 for experimental infection throughout the study. Before inoculation, the strain was grown in *Brucella* broth (Difco, Kansas City, Missouri, USA)

for 48 hours at 37° C with 5% $CO_2$. The organisms were washed 3 times and resuspended in physiologic saline.

*Experimental rats and inoculation*

We purchased 25 Sprague-Dawley (SD) rats, weighing 200 to 250 g, from a government-licensed commercial breeder. The rats were dewormed with piperazine and housed, handled, and fed according to standard humane protocols approved by the guidelines of Chonbuk National University, Jeonju, South Korea, for experimental animal use under the supervision of veterinarians. The animals were screened clinically, microbiologically, parasitologically, and serologically for evidence of disease. Any abnormalities precluded the animal from use in the experiment. Throughout the experiment, the animals were kept in a stringently hygienic, climate-controlled environment and provided with commercial feed and water *ad libitum*.

The female rats were kept with each male rat for mating and to see the vaginal plug. On day 1 after gestation, the vaginal plug was observed. Rats were classified into an infected group (*n*=15) and a control group (*n*=10). A suspension (500 μl) containing $1.0\times10^9$ colony forming unit (CFU) of *B. abortus* biotype 1 Korean isolate in physiological saline solution was injected subcutaneously to each of 15 rats of infected group at 18 days of gestation. Another ten rats received the same volume of saline as the sham control.

*Clinical and bacteriological examination*

Clinical parameters, such as body temperature, appetite, and thirst, were monitored daily. Appetite and thirst were assessed from the exact quantities of food and water consumed. The rats were also evaluated for pregnancy, abortion, premature birth, and any other adverse reactions.

After parturition the placenta were examined and 1 ml of heparinized blood by cardiac puncture in rats anesthetized with ketamine were also collected to isolate bacteria.

The rats were then euthanized and the spleen, uterus, and lymph nodes from infected (*n* = 15) and control (*n* = 10) SD rats were collected for gross, microscopic and bacteriological examination. All tissue samples were stored for 48 hours at 4° C before culture and bacteriological examination.

Blood was cultured at 37°C with 5% $CO_2$ for 3 days in tryptose soy broth (Difco) containing bovine serum. Subcultures were made on tryptose soy agar (Difco) to assess the colonial and morphologic characteristics of the bacterial growth [9]. Isolates were confirmed by PCR [10]. In tissue samples, the organism's identity was bio-chemically confirmed on the basis of $CO_2$ requirements, $H_2S$ production, and growth in the presence of thionine and basic fuchsin [9].

Microscopic examination

The cervical and mesenteric lymph nodes, spleen, uterus, and placenta from rats were fixed in 10% neutral-buffered formalin for at least 24 hours. Tissues were dehydrated in graded alcohols, cleared with xylene, and infiltrated and embedded in paraffin.

Tissues embedded in paraffin were cut at 4 to 6 micrometers and mounted on glass slides. Sections were stained with haematoxylin and eosin (H & E) and examined for microscopic changes under a light microscope [11].

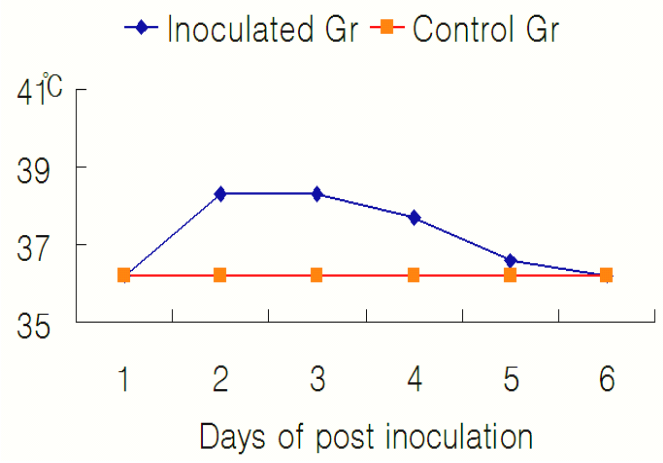
## Results

*Clinical signs*

All rats inoculated with *B. abortus* biotype 1 became lethargic, anorexic, and febrile within 24 hours. The highest rectal temperature was 38° C (within 72 hours). Since the normal temperature of the rat is 37° C to 38.7° C, these temperature changes were not considered significant (Figure 1).

No other adverse reactions or clinical signs were observed after inoculation. Anorexia and increased thirst were evident at Day 3 and became very severe at Day 5. Consumption normalized at Day 7.

**Figure 1.** Changes of rectal temperatures in *Brucella abortus* biotype 1 infected and control Sprague-Dawley rats.




*Reproductive signs*

The gestational periods and litter sizes were within the normal range in the infected and control groups. There was no evidence of abortion, premature birth, or stillbirth.

*Bacteriological characteristics*

Characteristic colonies of *B. abortus* biotype 1 were cultured from the blood as well as from the spleen, lymph nodes, placenta, and uterus tissue of the inoculated rats. The control rats remained culture negative.

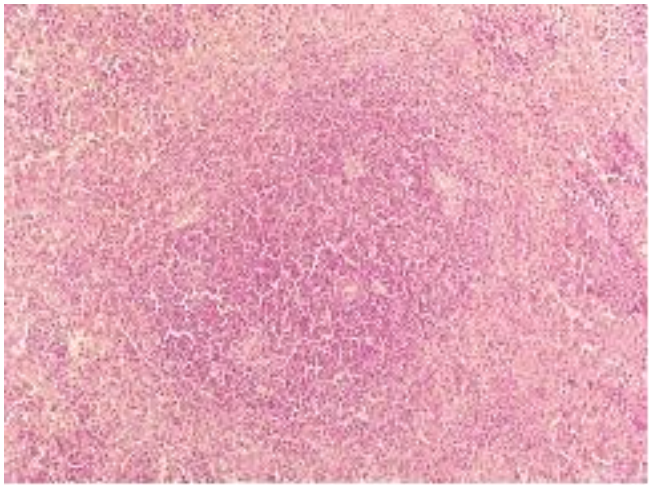
*Gross signs*

The gross signs of all the SD rats of the infected group included splenomegaly, metritis, enlargement of lymph nodes and placentitis, whereas there were no remarkable signs in the control group. The grossly cut surface of the enlarged spleen of infected SD rats had diffuse, random, slightly elevated foci 1-2 mm in diameter.

*Microscopic signs*

The endometrium of the uterus of rats infected with *B. abortus* biotype 1 was characterized by moderate, diffuse, but multifocally prominent accumulation of lymphocytes and macrophages in the superficial lamina. Neutrophils were also observed in some areas. In the spleen, there was diffuse congestion of the red pulp and diffuse infiltration of macrophages with increased giant cell numbers, and there was a prominent germinal center (Figure 2).

**Figure 2.** Spleen of *B. abortus* biotype 1 infected Sprague-Dawley rats. Prominent germinal center. H & E stain ×100.



In the placenta, there were areas of necrosis in the periplacentomal chorionic epithelium and adjacent interstitium accompanied by infiltration of underlying connective tissue with moderate numbers of macrophages. Severely affected placentas had vasculitis characterized by intimal and medial infiltrates of neutrophils and macrophages, intimal oedema, and fibrin deposition. In lymph nodes, there were infiltrations of macrophages and giant cells. There were vacuolated and engorged macrophages scattered in necrotic debris.

## Discussion

In this study, the female SD rats were used as a model for clinical findings and pathological changes after experimental infection at late stage of pregnancy with the bovine pathogenic strain *B. abortus* biotype 1. The findings from this study confirmed the generalized infection of SD rats by *B. abortus* biotype 1 with the spleen, lymph node, uterus and placenta being the main target organs as organisms were detected bacteriologically from these organs as well as in blood. Inflammatory cells were primarily macrophages, lymphocytes, and a lesser number of neutrophils. The prominent histopathological changes seemed to induce macrophages in the spleen as reported by other authors [12-13] and the increased number of macrophages in the spleen contributed to splenomegaly as reported by Palmer et al. [14]. Lymph nodes from all SD rats infected with *B. abortus* biotype I were markedly enlarged. The SD rats inoculated with *B. abortus* biotype 1 also developed lethargic, anorexic and febrile conditions, but control rats remained normal.

Abortion is a common outcome of *Brucella* infection in cows, swine and many other animals [15]. Nevertheless, Bosseray [16] challenged pregnant mice on days 3, 7, 11, or 15 of pregnancy with *B. abortus* strain 544 using several routes and observed neither abortions nor foetal deaths. In the present study, the female SD rats were injected experimentally at 18 days of gestation with bovine pathogenic strain *B. abortus* biotype 1 isolated in South Korea, and there were no abortions or foetal deaths. Brucellosis did not affect pregnancy in mice although placental colonization occurred as early as 5 minutes post inoculation [17]. In both cow and mice hosts *Brucella* colonization of the gravid reproductive tract can lead to severe placental damage, foetal infection and foetal death [18-19].

In this study, *B. abortus* biotype 1 did not affect the pregnancy of SD rats. All the SD rats delivered normally and did not show any abnormality of the fetuses or stillbirths. However there were necrosis in the periplacentomal chorionic epithelium of placenta and metritis in the rats infected at day 18 of pregnancy. Conclusively, it was observed that the Korean pathogenic isolate *B. abortus* biotype 1 does not induce abortion in the SD rats.

**Acknowledgements**

The authors thank Professor Dr. Ibulaimu Kakoma, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801, USA for initial review of this manuscript.

## References

1. Young JE (1995) An overview of human brucellosis. Clin Infect Dis 21: 283-290.
2. Moore CG, Schnurrenberger PR (1981) A review of naturally occurring *Brucella abortus* infections in wild mammals. J Am Vet Med Assoc 179: 1105-1112.
3. Oliakova NV, Antoniuk VI (1989) The gray rat as a carrier of infectious agents in Siberia and the Far East. Med Parasitol 3: 73–77.
4. Baek BK, Lee BO, Hur J, Rahman MS, Lee SI, Kakoma I (2005) Evaluation of the Sprague-Dawley rat as a model for vertical transmission of *Brucella abortus*. Can J Vet Res 69: 305-308.
5. Silva I, Dangolla A, Kulachelvy K (2000) Seroepidemiology of *Brucella abortus* infection in bovids in Srilanka. Prev Med 46: 51-59.
6. Chung JS, Cho YJ, Park CK (1988) Isolation and biotyping of *Brucella abortus* dairy cattle in Kyungbuk area, Korea. Korean J Vet Res 28: 339-343.
7. Park NC, Kim SY, Cho KH, Do JC, Kim YH, Shin SH, Cho MH, Oh KH, Kim WH, Kim JH, Jyeong JS, Kim SW, Kim BH (1998) Studies on the brucellosis in Kyeongbuk area. Korean J Vet Serv 21: 451-465.
8. Rahman MS, Baek BK (2007) Evaluation of polymerase chain reaction and comparison with serological tests for the diagnosis of brucellosis in Sprague-Dawley rats. Indian J Anim Sci 77: 118-120.
9. Alton GG, Jones LM, Angus RD, Verger JM (1988) Techniques for the Brucellosis Laboratory. Paris, France: Institut National de la Recherche Agronomique.
10. Ewalt DR, Bricker BJ (2000). Validation of the abbreviated *Brucella* AMOS PCR as a rapid screening method for differentiation of *Brucella abortus* field strain isolates and the vaccine strains 19 and RB51. J Clin Microbiol 38: 3085–3086.
11. Meador VP, Hagemoser WA, Deyoe BL (1988) Histopathological findings in *Brucella abortus* infected pregnant goats. Am J Vet Res 49: 274-280.
12. Riglar C, Cheers C (1980) Macrophage activation during experimental murine brucellosis. Inhibition of in vitro lymphocyte proliferation by *Brucella*-activated macrophages. Cell Immunol 49: 154-167.
13. Lauderdale TL, Jones SM, Winter AJ (1990) Response of murine T cell to antigen of *Brucella abortus* at sequential periods after infection. Immunol Infect Dis 1: 59-66.
14. Palmer MV, Cheville NF, Tatum FM (1996) Morphometric and histopathologic analysis of lymphoid depletion in murine spleens following infections with *Brucella abortus* 2308 or RB51 or an htr A deletion mutant. Vet Pathol 33: 282-289.
15. OIE (2000) OIE Manual of Standards for Diagnostic Tests and Vaccines. 4th edn., 12 rue de Prony, 75017 Paris, France.
16. Bosseray N (1980) Colonization of mouse placentas by *Brucella abortus* inoculated during pregnancy. Brit J Exp Pathol 61: 361-368.
17. Bosseray N (1983) Kinetics of placental colonization of mice inoculated intravenously with *Brucella abortus* at day 15 of pregnancy. Brit J Exp Pathol 64: 612-616.
18. Bosseray N (1982) Mother to young transmission of *Brucella abortus* infection in mouse model. Ann Rech Vet 13: 341-349.
19. Tobias L, Schurig GG, Cordes DO (1992) Comparative behaviour of *Brucella abortus* strain 19 and RB51 in the pregnant mouse. Res Vet Sci 53: 179-183.

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**Conflict of interest:** No conflict of interest is declared.